1955

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Paul R. Stowell
Yale University

Recommended Citation

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The C-reactive Protein Test in
Rheumatic Fever

by

Paul R. Stowell, B. S.
University of Utah, 1952

A Thesis Submitted to
the Faculty of the
Yale University School of Medicine
in Candidacy for the
Degree of Doctor of Medicine

Section of Preventive Medicine
and
Department of Pediatrics
1955

ACKNOWLEDGMENT

Appreciation is given for the teaching and inspiration of Drs. John R. Paul and Milton J. E. Senn.

The guidance, scholarly attitude, and exactness of Dr. Paul L. Boisvert have made this study a fruitful experience for the author. Valuable suggestions were given by Dr. Ruth Whittemore. Miss Joan B. Brockett facilitated the laboratory work.

The author is deeply indebted to and appreciative of his wife for her encouragement and assistance.

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THE C-REACTIVE PROTEIN TEST IN RHEUMATIC FEVER

I. INTRODUCTION

The C-reactive Protein determination has in recent years aroused much interest as a clinical test in rheumatic fever (4, 11, 39, 49, 50). C-reactive Protein (CRP) is an alpha-globulin found in the serum of patients with many infectious diseases and inflammatory processes, including rheumatic fever, subacute and acute bacterial endocarditis, pneumonia, certain viral diseases, most neoplastic diseases, abscesses, myocardial infarction, and rheumatoid arthritis. This protein is not present in the serum of well individuals.

It should be mentioned at this time that published data indicate that the C-reactive Protein test is a most sensitive index of the presence or absence of rheumatic activity (39, 49). C-reactive Protein (CRP) is usually present during the active stage of rheumatic fever, and it is generally not present during the inactive disease. For many years, an elevated erythrocyte sedimentation rate has been used as an important laboratory aid in the diagnosis of active rheumatic fever. Therefore the relatively new CRP test will be compared with the ESR in 66 examples of rheumatic fever. In this study the precipitin technique of Swift (41) was used, and the reagent is an antiserum prepared commercially by Schieffelin and Company.

The results of the CRP test in upper respiratory infections, during menstruation, and in pregnancy are also considered in this study. Upper respiratory infections and menstruation occur rather commonly and may complicate the evaluation of activity in patients with a past history of rheumatic fever. Occasionally the clinician observes patients with rheumatic carditis and pregnancy. It is very

important to know if these three conditions, i.e. upper respiratory infections, menstruation, and pregnancy interfere with the interpretation of the CRP test.

Several methods have been developed to detect the presence of C-reactive Protein. These methods have given different results in viral, neoplastic, and acute renal diseases and similar results in rheumatic fever and most bacterial infections. Recent studies show that the CRP test may be a valuable aid in the diagnosis of most acute and a few chronic diseases. A review will be made of the various determinations, differences of results, and range of usefulness of the CRP test.

II. REVIEW OF THE LITERATURE

The C-reactive Protein was first discovered in 1930 by Tillett and Francis (43). Prior to this, Tillett, Goebel, and Avery (42) isolated from the Type XXVII Pneumococcus a non-protein fraction which was different from the type-specific pneumococcal capsular polysaccharides previously identified. This non-protein fraction was a somatic polysaccharide, and it was named the "C-polysaccharide". It caused capsular swelling of at least Type I, II, and III Pneumococci, while the type-specific capsular polysaccharides reacted only with their homologous antiserums.

Tillett and Francis in 1930 demonstrated that the serums from individuals acutely ill with lobar pneumonia possessed the capacity to precipitate in high titers the C-polysaccharide just described. The substance which was present in acute phase serums was later named the C-reactive Protein. Shortly following the crisis in pneumonia, CRP was no longer demonstrable. Using C-polysaccharide as an antigen, Tillett and Frances also demonstrated CRP in the serum of patients with lung abscess, rheumatic pericarditis, osteomyelitis (Staphylococcus aureus), subacute bacterial endocarditis, and tuberculosis. No CRP was found in the serum of patients with malaria, liver cirrhosis, anemia (unknown etiology), measles, and chicken pox, nor in the serum of normal individuals. This study was the first to indicate that there is a factor (CRP) present in the acute phase serum of patients with infectious diseases which is not present in normal serum.

In 1933 Ash (3) studied 100 hospital patientsfor the presence of CRP using a 1:4,000 dilution of acute phase serum and C-polysaccharide in a precipitin test. She found that CRP could be demonstrated in diseases due to gram-negative as well as gram-positive organisms. He also found CRP in the serum of patients with bronchitis, septicemia, Still's disease,

typhoid fever, pyelitis, rheumatic fever, and upper respiratory infections. However, in many of these conditions the tests became negative when the patient was in the convalescent phase. In upper respiratory infections, for example, CRP was present in four of five febrile patients, and in four without fever but with other signs of upper respiratory infection. Seven patients with a recent history of upper respiratory infection who were well at the time of examination were negative for CRP. CRP could not be demonstrated in two patients with syphilis nor in twelve with non-infectious diseases.

Abernathy and Francis in 1937 (1) continued the study of the CRP by testing the serum of patients with acute and convalescent pneumonia. CRP was present in 100% of the acute cases (46 patients), and absent in 97.4% of convalescent cases (39 patients). They gave further proof that CRP was present in active rheumatic fever, bacterial endocarditis, empyema, and septicemia, and that it was not present in inactive rheumatic fever, malaria, and in nineteen normal persons. It was suggested that there was some correlation of the white blood count, the temperature, and the presence of CRP.

Abernathy and Avery, and MacLeod and Avery in 1941 (2, 25) first demonstrated the protein nature of the CRP. It was precipitated from the serums of acutely ill patients with a 50 to 75% saturated solution of ammonium sulfate followed by dialysis of the precipitated fraction against tap water. Sodium sulfate, however, was more effective because it produced larger quantities. The CRP would not form a precipitate unless calcium ions were present in solution. These investigators suggested that the CRP was an albumin. They did immunological studies which demonstrated the difference between CRP and antibodies of the gamma globulin variety. They noted that CRP lacked specificity, that it was

a much smaller protein than the gamma globulins, and that high titers of CRP occurred within 18 to 36 hours after the onset of an acute disease. These titers were not present in early convalescence.

MacLeod and Avery (1941) also demonstrated that the CRP was highly antigenic upon injection into rabbits and that the antiserum thus prepared reacted with the CRP but not with the proteins of normal human serum. Immunological specificity was shown by both complement fixation and precipitin tests. The antigen-antibody reaction of CRP antiserum derived from rabbits plus CRP from human serum was shown to be a much more sensitive test than the earlier C-polysaccharide and CRP precipitin method (26).

Lofstrom (22) produced a nonspecific capsular swelling test of the pneumococcus by the addition of acute phase serum to these organisms. He suggested that the agent which produced capsular swelling was an alpha or beta globulin. The serums of 400 hospital patients were studied, and he found that in most acute bacterial infections a substance was present in the serum which was capable of producing capsular swelling. The patients were well into the convalescent phase if no such substance was formed. Poliomyelitis, smallpox vaccination, cardiovascular diseases and about half of various tumors tested, on the other hand, were not capable of inciting the production of this pneumococcal capsular swelling substance (CRP). Lofstrom was the first to suggest that this test did not always correlate well with the white blood count and the erythrocyte sedimentation rate. In 1944 (23) he described the similarity of the results of the acute phase serum-C-polysaccharide test and the acute phase serum-pneumococcal capsular swelling test. He concluded that the tests were positive in most bacterial infections, in conditions which produced destruction of tissues, and in response to the injection of colloidal sulfur.

Up to this point with workers in at least two countries approaching this problem from somewhat different points of view, the question was raised whether the antibody produced by C-polysaccharide in monkeys or humans was the same as the CRP in human acute phase serum (2, 26). Both CRP and C-polysaccharide antibody had been shown to precipitate in the presence of C-polysaccharide. Perlman, et al (31) found that the two substances were not the same inasmuch as the C-polysaccharide antibody and the CRP were different globulins, the former being a gamma globulin and the latter an alpha globulin. This he demonstrated with electrophoretic studies. Also precipitin titrations using a photoelectric turbidometer produced a broader curve with the CRP-C-polysaccharide precipitate than with the C-polysaccharide antibody-C-polysaccharide precipitate.

Hedlund (16) agreed with Lofstrom that the acute phase protein in serum which produced nonspecific capsular swelling of the pneumococcus and the CRP were the same substance. Using the capsular swelling test he extended the study of the CRP to include over 1,000 individuals with a wide variety of illnesses. Those results which apply to this investigation include:

	CRP Test*	
	Positive	Negative
Rheumatic fever	28	0
Congestive heart failure	9	4
Acute upper respiratory infections	13	23
Acute tonsillitis	2	0
Sinusitis	6	2
Influenza	3	6
Bronchial asthma	3	12
Acute pneumonia	252	2
Pregnancy	0	25
Totals	316	74

* Pneumococcal Capsular Swelling Method

Anderson and McCarty in 1950 (4) studied the CRP test in 45 cases of rheumatic fever and they were of the opinion that a positive CRP test was a very reliable indicator of rheumatic activity. They studied the results of the white blood count, the erythrocyte sedimentation rate, and the CRP test, and compared these findings with the clinical condition of the patients. There was some correlation between the results of the erythrocyte sedimentation rate and the CRP test. However in the convalescent stages of rheumatic fever the CRP test was usually negative before the erythrocyte sedimentation rate had diminished to a normal value. They were convinced that the CRP was not the same agent that induced fluctuation of the erythrocyte sedimentation rate. The fibrinogen level of the plasma is considered to be the most important factor in erythrocyte sedimentation rate changes (8), while the CRP, as has been mentioned, is an alpha globulin.

To determine which of the several methods described was the most sensitive for CRP determination Wood and McCarty in 1951 (48) compared the CRP antiserum (MacLeod and Avery 1941) and the C-polysaccharide as test substances. They used the quantitative technique of spectrophotometry and demonstrated a range of CRP from zero in normal serum to as high as 0.33 mg/cc in acute rheumatic fever serum. Their conclusions were that the CRP antiserum test produced visible precipitation when the quantity of CRP in the serum was as low as 0.01 mg/cc while the C-polysaccharide test was not reliable when the CRP was below 0.1 mg/cc. The CRP antiserum was considered to be at least ten times more sensitive in the lower ranges and therefore it should be more useful in reflecting changes in activity in rheumatic fever or other inflammatory conditions. In this same study the serums of 68 patients with viral hepatitis were tested. Using the CRP antiserum they found 30 to contain CRP in a sufficiently high concentration to form visible precipitate. The tests were

graded from 0 to 6-plus depending upon the height of the column of precipitate in a capillary tube. The highest reading in these cases was 2-plus. They agreed with previous investigators that the CRP was much more likely to be present in the acute phase of an illness; i.e. four serums were positive and one was negative during the first to the seventh day of viral hepatitis; eight were positive and three were negative from the eighth to the fourteenth day of illness; and only one of nine was positive when the duration was 60 days or over.

Further investigators of the CRP studied serums drawn every few days from rheumatic fever patients. They cautioned that cortisone and/or ACTH affect the results of laboratory tests used in the study of rheumatic fever patients. Ziegra and Kuttner, and others, (5, 28, 50) demonstrated that these patients frequently showed abnormal laboratory tests during and following withdrawal of ACTH or cortisone. The clinical activity diminished and the white blood count, the erythrocyte sedimentation rate, and the CRP fell during hormone therapy. Upon withdrawal of these harmones however, there tended to be a rebound phenomenon. This lasted for several days and then the WBC, ESR, and CRP returned to normal levels again unless a definite exacerbation of the disease occurred. It is interesting that the ESR diminished more slowly than the level of the CRP and climbed more slowly during the rebound phase. The authors believed that the CRP levels and the erythrocyte sedimentation rate were actually higher for a few days following cessation of therapy than they would normally be had there been no hormonal therapy. Fearnley and Bunim (7) found a decrease in the fibrinogen and other serum protein levels in normal patients following administration of ACTH. Therefore, one would expect a drop in the erythrocyte sedimentation rate and indeed finds this to be true following such therapy.

What the mechanism is for the CRP diminution with hormone therapy is not definitely known (11). Fearnley and Bunim (7) suggested that the absence of CRP or a normal erythrocyte sedimentation rate may not be reliable indicators of inactivity if hormonal therapy is being given. Anderson and McCarty state that the CRP is a very useful test even during hormone therapy because if the CRP is still positive one can assume that inadequate levels of hormone are being given.

The CRP* has also been studied in rheumatoid arthritis by Hill (17) in England. Using the C-polysaccharide as a test reagent he tested the serum from 139 patients. Fifty-six of 66 clinically active cases gave positive results; seven of 22 who were mildly active were positive; and all of ten who were inactive were negative. There were also 41 other rheumatoid patients whose serum contained large quantities of C-polysaccharide antibody.** He stated that C-polysaccharide antibody was not the same substance as CRP and that some of the earlier workers may have been demonstrating the presence of C-polysaccharide antibody rather than C-reactive Protein. Thus earlier studies should be repeated using more sensitive and specific methods to find the range of conditions in which the CRP is positive. Hill compared the results of the CRP test with the erythrocyte sedimentation rate (Westergren method). The CRP test correlated better than the ESR with the clinical condition of the patients studied.

As has been stated, one cannot be certain from a review of the literature that positive CRP tests always denoted that CRP was in the serum. Because pneumococci and C-polysaccharide (an antigen from the pneumococci) were used as test reagents, a C-polysaccharide antibody of an immune or globulin variety could have been present in certain cases

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* CRP - an alpha globulin
** C-poly. antibody - a gamma globulin

and is sometimes present in normal human serum. Hill summarizes what has previously been described on this problem as follows:

> "The reaction of C-polysaccharide with acute phase serum differs in several ways from classical immune precipitation. Thus it does not occur in the absence of calcium ion, and on salt fractionation of the serum the reactive protein is found in the albumin portion, though electrophoretic studies suggest that it is an alpha globulin. C-polysaccharide antibody which also occurs in human serum (Heidelberger and Anderson) reacts with its antigen in the absence of calcium; it is found in the globulin fraction on salt fractionation and has electrophoretic mobility of gamma globulin." Thus we see that further

studies with CRP antiserum are indicated. Certainly the CRP antiserum first developed by MacLeod and Avery, and quantitatively studied by Wood and McCarty, obviates such confusion. This reagent is a specific antibody developed by injection of CRP itself into rabbits.

Other conditions which have recently been studied in which CRP is claimed to be present are the post-commissurotomy syndrome and acute myocardial infarction. In the former study Elster et al (6) found no significant changes in the ASLO titer, the ESR, or the WBC count in sixteen patients who developed a clinically demonstrable post-commissurotomy syndrome. The CRP, however, was present in fourteen of these sixteen patients. In myocardial infartion, Kroop et al (20) suggested the use of the CRP test as a diagnostic aid. Of eight patients with coronary occlusion and myocardial infarction <u>with necrosis</u>, the CRP test was positive in all. No CRP was present in six patients with coronary insufficiency <u>without</u> necrosis. In comparing these results

with the corrected erythrocyte sedimentation rate they found that in patients with myocardial infarction with necrosis, all had ESRs of 21 mm. or over (Wintrobe method). In patients with coronary insufficiency without infarction in which all the CRP tests were negative, four ESRs were over 15 mm. and two were 20 mm. or over. These findings suggest that the erythrocyte sedimentation rate may be elevated due to other factors than those immediately apparent.

A recent study using highly potent antiserum was reported by Good in 1952 (11). The CRP test was positive by this method in 100% of 36 patients with exudative rheumatic fever, and negative in 100% of 28 convalescent and 14 inactive patients. C-reactive Protein was usually not present in patients with Sydenham's chorea unless other manifestations of rheumatic activity were also present. Good was able to demonstrate a positive test in acute and a negative test in chronic glomerulonephritis. This was in contrast to Hedlund (16) who found only negative results for both groups. The difference here is undoubtedly due to the more sensitive antiserum technique used by Good. Most but not all patients with collagen diseases had CRP in their serum. It is interesting that most neoplastic diseases tested in children produced positive CRP tests; i.e. acute lymphatic leukemia, lymphosarcoma, Hand-Schuller-Christian's disease, neuroblastoma, Wilm's tumor, malignant teratoma of the ovary. A benign mesenteric cyst and one benign teratoma of the ovary were negative. Infants and children were shown to be capable of producing CRP in response to inflammation. This included one premature infant.

A study in which the presence of the CRP has been suggested as a guide to the treatment and management of rheumatic fever was done by Stollerman, Glick, et al (39) in 1953. They performed serial CRP tests

with serums of 62 patients in various stages of rheumatic fever, and during treatment. When the cases were classified as "frank", "low grade", "doubtful", "pure chorea", they found the following results:

Rheumatic Activity	Number of Patients	Positive	Negative
Frank	35	34	1
Low grade	11	10	1
Doubtful	11	6	5
"Pure" chorea	5	0	5

They gave further substantiation to the Anderson and McCarty (4) and Good (11) reports by stating that the presence of CRP in the serum of a patient suspected of having rheumatic fever was a reliable indicator of rheumatic activity. They suggested that if the CRP was positive during hormone therapy it indicated inadequate treatment, and that the disappearance of the CRP without hormone therapy was a reliable indication of a good prognosis although it did not preclude a future exacerbation. It was also found that certain rheumatic manifestations such as chorea, subcutaneous nodules, erythema marginatum, and Aschoff bodies in the auricular myocardium may be present individually with a negative CRP test. They suggested that the CRP test was the most consistently positive test in active rheumatic fever, and that false positives did not occur as may be true with the ESR and other laboratory tests.

Wood and McCarty (49) have recently reviewed laboratory aids in rheumatic fever, and they reiterated their former conclusions that the CRP was more sensitive than the ESR and that it was a more desirable test in evaluating rheumatic activity. Their major thesis was that the CRP rises and falls more quickly than the ESR and that there were no false positives with the CRP as long as other definite illnesses could be ruled out. They also stated that the normal range for the ESR in

children had never been completely established, and therefore the CRP was a preferable test because it was either definitely positive or negative. No normal range or correction factors were required for this test.

In Summary: The literature indicates

(1) The use of specific rabbit antiserum is the most delicate method for the detection of CRP.

(2) C-reactive Protein is not present in the blood of normal individuals.

(3) The CRP test is a sensitive indicator of infection and inflammation.

(4) CRP appears promptly at the onset of an illness and disappears early in convalescence.

(5) The presence of CRP during the active stage of most viral, bacterial, collagen, and neoplastic diseases indicates its non-specificity.

(6) The CRP test has been studied most extensively in rheumatic fever, and the result is felt to be a reliable indication of the presence or absence of rheumatic activity.

III. OBJECTIVES

This investigation was undertaken for the following reasons:

(1) It is important to substantiate the observations previously described as they relate to rheumatic fever and rheumatic activity since at this time there is no single laboratory test which is consistently at least as reliable as total clinical judgment.

(2) The sensitivity and non-specificity of the test demand further evaluation. If the test is so <u>sensitive</u> and <u>non-specific</u> that it is affected by such commonly present conditions as mild upper respiratory infection, menstruation, or pregnancy, then it may lose some value as a diagnostic aid in rheumatic fever. This will be investigated.

(3) A study of the CRP and the ESR in rheumatic fever as well as in menstruation will compare a relatively new test with a well established test and certain problems in the use of the ESR and the CRP will be discussed.

IV. CLINICAL MATERIAL AND LABORATORY METHODS

Rheumatic Fever Patients

The most recently suggested requirements for the diagnosis of rheumatic fever are those of Jones (19) as modified by the American Council on Rheumatic Fever and Congenital Heart Disease.* Symptoms, signs, and laboratory data are divided into major and minor criteria.

In this current study a few minor changes in this standard were made for the selection of case material. Changes usually resulted from insufficient detail of the recorded data; this, however, did not appreciably alter the selection of these patients.

The requirements for the diagnosis of active rheumatic fever in a patient were: 1) The presence of two major criteria, or 2) the presence of one major and at least two minor criteria. The major criteria chosen for this investigation were carditis, polyarthritis, subcutaneous nodules, chorea, and a "rheumatic" rash. (Erythema marginatum was suggested by the modified Jones criteria). The rashes in this group were not always defined clearly, but were counted if the clinician noted that a significant rash was present. The minor criteria were fever, arthralgia, prolonged P-R interval in the electrocardiogram, an elevated Antistreptolysin titer (250 units and above), and a definite past history of rheumatic fever. One of the following may be considered as an additional minor criterion of rheumatic fever according to the modified Jones plan: an elevated erythrocyte sedimentation rate, an elevated white blood count, or a positive C-reactive Protein test. Because the CRP test was under study in this investigation, it was not considered as a minor criterion in the selection of the cases. The WBC,

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*Unpublished data (Note Appendix).

although recorded in many instances, was not always available so it was not included. The ESR was counted as a minor criterion.

The requirements for the diagnosis of inactive rheumatic fever were a past history of rheumatic fever and the absence of all other major and minor criteria with the following exceptions: 1) A cardiac murmur was present in twelve of these patients, but it was unchanged since the previous examination, or, if changed it had decreased in intensity. The heart sounds and exercise tolerance were good in each patient; 2) The Antistreptolysin titer was elevated in thirteen instances, but these titers were either lower or stabilized in each case when compared with previous Antistreptolysin titers; and 3) Electrocardiograms concomitant with the other data were not available in two instances. In one patient there was a stable P-R interval of 18 months, and the last electrocardiograms of the second patient were done seven months earlier.

Rheumatic fever patients with questionable activity were not assignable to the active or the inactive group. These were classified as rheumatic fever patients - activity not established.

The records of 75 patients who had been admitted to the Grace-New Haven Community Hospital or the New Haven Rheumatic Fever Clinic during 1953 and 1954 were studied. Clinical and laboratory data were abstracted from these records. For the purposes of this study it was felt necessary that certain minimum standards for such data should be met. For each selected case, the blood samples for a CRP test and an ESR were drawn within three days of each other. The day of the clinical examination coincided in a similar way with the date of the laboratory tests. Twenty-five cases were not included since the laboratory and clinical data, although complete, were not sufficiently synchronous. The remaining

50 patients were classified as _active_, _activity not established_, and _inactive_ according to the criteria just described. In sixteen instances patients were assigned to the active category in the early phase of their illness and to the inactive status later.

In 31 instances the CRP test had not been done at the time of the other determinations. However, frozen samples of serum were available which had been drawn at the appropriate time. These serums had been frozen in the Streptococcus Laboratory for a period of up to two years. They were originally used for Antistreptolysin and other hemolytic streptococcal tests. CRP tests were done using these frozen serums.

Menstrual Patients

Menstruation for this study included active menstruation, the two days prior to menstruation, and the two days following the cessation of menstruation. Young girls and women were selected to represent each of these three related periods. Blood was drawn for CRP and ESR tests from sixteen healthy girls at the Southbury Training School, Southbury, Connecticut.* These girls represented the ages of ten to twenty years, inclusive. They were clinically free of recent illness. Blood samples were also drawn from four healthy adult females. This latter group ranged in age from 25 years through 40 years. Serial samples were drawn from two of the latter patients to determine if any particular portion of the menstrual period was more likely to alter the CRP test results.

Patients With Upper Respiratory Infection

An upper respiratory infection included one or more of the following: 1) otitis media, 2) rhinitis, 3) objective cough, 4) pharyngitis, 5) laryngitis, and 6) cervical lymphadenopathy. Fifteen patients with

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*Dr. Herman Yannet and his staff were of help in this portion of the study.

upper respiratory infections who entered the emergency room of the Grace-New Haven Community Hospital in January, February, and March of 1955, were examined. Three additional patients from the Southbury Training School were selected. These children were four to sixteen years of age. They were examined for the above criteria and to exclude other clinical illnesses. Recorded data included the day of illness, rectal temperature, and the findings on physical examination. Blood was drawn for an ESR, WBC, and CRP studies. Nose and throat cultures were taken in a few instances.

Pregnancy Patients

Thirty--six pregnant women were selected at random from the prenatal clinic of the Grace-New Haven Community Hospital and from the delivery suite of the Memorial Unit of the Hospital. A five to ten cc. sample of blood was drawn from each woman for CRP determinations. These women represented the three trimesters of pregnancy. Six patients in the last trimester were in labor. The hospital records of these women were studied and any illness or abnormality of pregnancy was noted.

LABORATORY METHODS

C-Reactive Protein Test

Ten cc. of blood was drawn into a dry sterile syringe from the antecubital vein of each subject. A five cc. sample for CRP determination was transferred to a sterile 13 X 100 mm. test tube. The tube of blood was centrifuged at 2500 r.p.m. for ten minutes, and the serum was transferred to a similar but more permanently labelled sterile test tube. The liquefaction of frozen serums was done at room temperature.

Materials used for CRP Test

1) CRP Antiserum (Schieffelin) 1cc. vials containing approximately 1 cc. CRP Antiserum with 1:10,000 ethyl-mercuri-thiosalicylate as a preservative and antiseptic agent. (41)
2) Patient's serum.
3) Capillary tubes--7-8 cm. x 0.7 mm.
4) Capillary tube rack with plasticine-filled groove. (41)

The general method for the precipitin test in this study is that described by Swift for the typing of hemolytic streptococci. (41). For each CRP test the tip of a small capillary tube was inserted in the CRP antiserum and the antiserum was allowed to run up into the tube for a distance of three to four centimeters. The tip of the capillary tube was then inserted into the patient's serum, and by gently tilting the capillary tube and the test tube towards the horizontal, three to four centimeters of the patient's serum was drawn into the capillary tube. Caution was taken to prevent bubbles from forming between the two liquids. This was done by holding the capillary tube partially upright at approximately 15 degrees above the horizontal position while moving it from the CRP antiserum vial to the tube of patient's serum. It is important that the order described be followed to prevent contamination of the CRP antiserum. The tip of the capillary tube was then pressed carefully into plasticine to thoroughly seal the lower end. The tube was placed in a vertical position in the groove of the capillary tube block. A series of five to ten tests at a time was performed. The block with tubes was placed in an incubator at 37 degrees C. for two hours. At the end of this time the rack was removed from the incubator and placed overnight in a refrigerator at 4 degrees C. Readings were made the following day. Each capillary tube was carefully removed from the block and cleaned with a tissue moistened in alcohol. The

readings were made against a dark background with a source of light shining at an oblique angle from behind the tube. A white precipitate formed in positive tests and it was measured carefully. In making these readings the tubes were examined from the top to the bottom of the liquid column with the aid of a small magnifying hand lens. Results were graded as _negative_ - no visible precipitate; _trace_ - a few small particles; and for readings of _1-, 2-, 3-, 4-, 5-, and 6- plus_ a millimeter scale was held adjacent to the capillary tube and the actual height of the column was measured. Frequently the precipitate was scattered in small portions in various parts of the tube. In these cases some allowance for packing was made, but each portion of the precipitate was measured and the results of each measurement were added for the final reading (Figure 1). If an answer was felt to include a fraction of a millimeter, the smaller whole number was used in each case, i.e. 4½ mm. was called 4 mm. or 4-plus. Six-plus was considered a maximum reading and any reading of 6 mm. or more was recorded as six-plus. Unknowns read by several observers to check the accuracy of this method gave answers always within one millimeter of each other, i.e. 3-plus was read as 2½ mm. to 3½ mm. by these readers.

Erythrocyte Sedimentation Rate

The method of Wintrobe was used (26). Five cc. of blood for each determination was drawn and placed in a bottle containing six mg. of ammonium oxalate and four mg. of potassium oxalate. The blood sample and oxalate were mixed, a Wintrobe hematocrit tube was filled to the 0 mm. mark, the tube was supported in a vertical position, and a reading was made at the end of 60 minutes. To determine the packed cell volume a second reading was made after centrifugation at

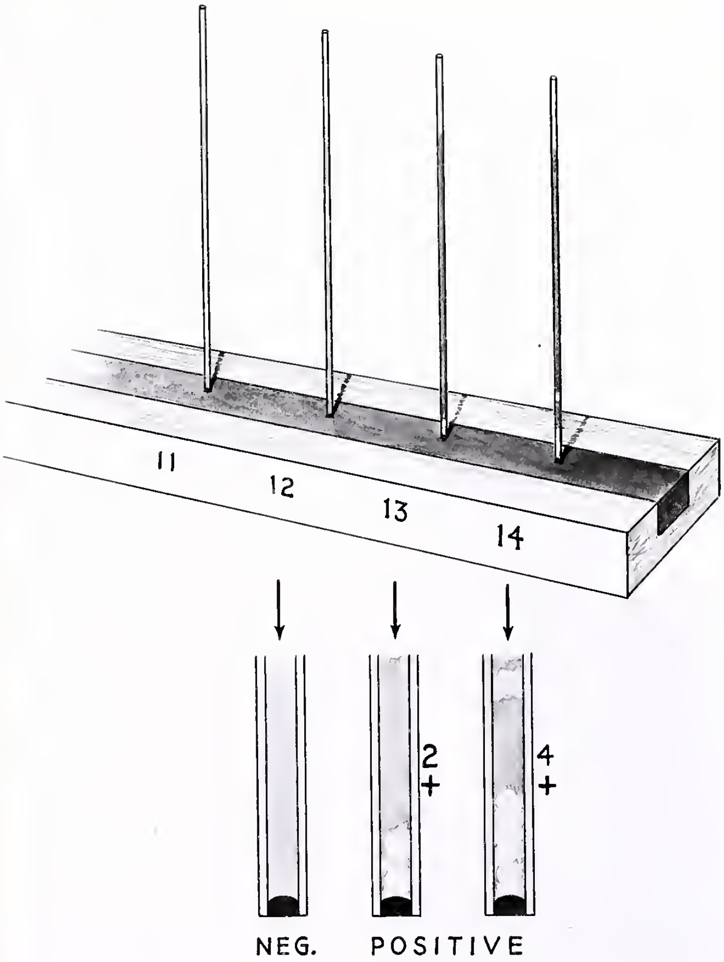

Figure 1. The Results of the C-reactive Protein Test

The results and set-up of the CRP test are shown. Note the block with plasticine-filled groove which supports the capillary tubes. Detailed drawings of tubes 13, and 14, show the fluffy white precipitate found in positive tests; the two-plus and four-plus denote the approximate amount present. Two-plus and four-plus represent 2mm. and 4mm., respectively. A negative test is also shown for comparison (tube 12).

3,000 r.p.m. for 30 minutes. The Wintrobe-Landsberg (45) correction chart was used to adjust hematocrits to 47 mm. for males and to 42 mm. for females. The normal range for this investigation in all instances was: males - 0 through 10 mm.; females - 0 through 15 mm. Any reading above these normals was classified as <u>elevated</u>.

V. OBSERVATIONS

Table I presents clinical and laboratory data for thirty-three active rheumatic fever patients. The ages represented are three through 46 years. There are fifteen females and eighteen males. The major and minor criteria have been defined on page fifteen. The letters A, B, C, D, represent various signs of carditis; A) a significant systolic or diastolic murmur (note page 39); B) cardiac enlargement; C) pericarditis; and D) congestive heart failure. Twenty-five of the 33 patients had carditis. Arthralgia and a prolonged P-R interval, (although recorded when present) were not officially counted as criteria in the selection of case material if polyarthritis and carditis, respectively, were present. A plus-minus in the Prolonged P-R Interval column denotes a borderline P-R interval. Eighteen patients had a prolonged or borderline P-R interval. The Antistreptolysin (ASLO) titer was above 250 units (upper limit of normal) in all but four of 33 determinations recorded. Patients J.G., R.W., P.P., and J.C. (Nos. 25, 28, 29, and 32) had ASLO titers of 125, 75, 175, and 200 units. No followup ASLO titers were available for these patients. For J.G., P.P., and J.C. the CRP test was positive and the ESR was elevated. R.W. had a corrected ESR of only 9 mm. but a strongly positive CRP test. No carditis was present in R.W. In the group of active rheumatic fever patients, the CRP test was positive in 30 and negative in three. By comparison the ESR was elevated in 32 patients. Each ESR was corrected to a packed erythrocyte volume (hematocrit) of 47 mm. for males and 42 mm. for females. In this series the CRP test does correlate well with the ESR as an aid in the determination of rheumatic activity. Only four exceptions to this correlation occurred

TABLE I. ACTIVE RHEUMATIC FEVER PATIENTS - CLINICAL AND LABORATORY DATA

No.	Patient	Age	Sex	MAJOR CRITERIA								MINOR CRITERIA				ASLO TITER	ESR	CRP TEST
				CARDITIS *				Poly-arthritis	Chorea	Subcutaneous Nodules	"Rheumatic" Rash	Fever	Arthralgia	** Prolonged P-R Interval	Past History of Rheum. Fever			
				A	B	C	D											
1	P.B.	13	F	yes								yes	yes	±		2350	54	4+
2	P.H.	9	F	yes	yes			yes			yes	yes	yes			625	70	3+
3	B.A.	5	F	yes				yes	yes			yes	yes			1475	19	4+
4	R.S.	13	M					yes				yes	yes	yes		3125	13	6+
5	M.B.	7	F	yes				yes				yes	yes			1700	44	2+
6	R.P.	10	M	yes				yes				yes	yes			600	33	2+
7	J.A.	8	F	yes								yes	yes			575	25	3+
8	W.C.	12	M	yes								yes	yes	yes		1250	30	0
9	R.G.	17	M					yes				yes	yes		yes	275	29	6+
10	M.R.	40	M	yes						yes			yes	±	yes	2000	32	0
11	R.M.	9	M	yes				yes				yes	yes	yes		2250	37	3+
12	M.C.	10	F	yes	yes	yes	yes					yes		yes		1300	40	5+
13	J.P.	14	M					yes					yes			525	33	3+
14	J.M.	13	M	yes								yes	yes			1750	33	4+
15	B.D.	9	F	yes	yes							yes		±		800	25	3+
16	R.D.	7	M	yes	yes					yes		yes		yes	yes	1125	16	3+

17	E.S.	33	F					yes		yes	yes	yes	yes			575	53	6+
18	A.R.	33	M					yes				yes	yes	±		500	23	6+
19	G.C.	11	M	yes				yes				yes	yes	yes		1500	28	6+
20	D.D.	10	F					yes				yes	yes	yes		975	48	6+
21	J.K.	11	F	yes				yes				yes	yes	yes		1000	50	5+
22	E.G.	46	M	yes	yes			yes			yes	yes	yes			275	34	5+
23	F.A.	3	M	yes	yes			yes			yes	yes	yes	±		320	29	6+
24	J.L.	10	M	yes								yes		±	yes	725	28	4+
25	J.G.	4	F	yes				yes					yes			125	35	2+
26	J.C.	4	M	yes	yes			yes			yes	yes	yes	yes		2110	33	6+
27	G.W.	9	M					yes				yes	yes	yes		420	31	4+
28	R.W.	20	M					yes				yes	yes		yes	75	9	3+
29	P.P.	38	F	yes	yes									yes	yes	175	42	6+
30	S.D.	13	M	yes				yes					yes			925	31	4+
31	E.C.	8	F	yes				yes				yes	yes	±	yes	425	20	4+
32	J.C.	6	F	yes											yes	200	18	1+
33	C.A.	11	F	yes	yes											1200	49	0

* A - A significant systolic or diastolic murmur.
B - Cardiac enlargement by x-ray.
C - Pericarditis.
D - Congestive heart failure. (For further details see Appendix)

** ± - Borderline P-R Interval in the electrocardiogram.

Patients W.C., M.R., and C.A. (Nos. 8, 10, and 33) had negative CRP tests, while they had elevated ESRs and elevated ASLC titers. M.R.'s titer had risen from 1600 units to 2000 units in five days; there was only one determination for W.C. and the titer for C.A. dropped to 465 units in about three months. Two patients had carditis. None of these three had polyarthritis. By contrast patient R.W. (No. 28) had a 3-plus CRP test with a normal ESR of 9 mm., an ASLO titer of 75 units, and no carditis. It is interesting that the CRP test was moderately to strongly positive (3-plus to 6-plus) in 26 of the 29 positive tests.

Table II follows the form of Table I and refers to 28 inactive rheumatic fever patients; these were three through nineteen years of age. Cardiac murmurs, although present in thirteen instances were not felt to represent active carditis. This column is labelled "Heart Disease". These murmurs had either diminished in intensity or remained constant since the previous examination. None of these thirteen patients had chorea or subcutaneous nodules. The asterisks in the P-R interval column represent instances wherein an electrocardiogram was done from two to nine months prior to the recorded examination. R.S. (No. 16) had only one EKG done; B.C. (No. 11) had adequate evidence of a relatively stable P-R interval for at least eighteen months. Fifteen ASLO titers were within normal limits while eleven were still elevated; the latter were decreased from previous determinations. The CRP test was negative in 24 of the 28 patients classified as inactive. The four positive reactions in the remaining four patients ran from trace to 1-plus amounts of precipitate. It is not clear why these four were positive. Three were definitely less positive than on previous determination. The fourth

TABLE II. INACTIVE RHEUMATIC FEVER PATIENTS - CLINICAL AND LABORATORY DATA

No.	Patient	Age	Sex	MAJOR CRITERIA								MINOR CRITERIA						
				HEART DISEASE *				Polyarthritis	Chorea	Subcutaneous Nodules	"Rheumatic" Rash	Fever	Arthralgia	Prolonged P-R Interval	Past History of Rheum. Fever	ASLO TITER	ESR	CRP TEST
				A	B	C	D											
1	D.R.	8	F												yes	50	20	0
2	R.T.	10	M	yes											yes	100	13	1+
3	S.D.	13	M	yes											yes	250	4	0
4	D.J.	11	F	yes											yes	400	5	0
5	K.D.	6	F												yes	50	5	0
6	C.R.	11	F												yes	75	21	0
7	L.K.	9	M												yes	200	6	0
8	J.K.	10	M												yes	275	23	0
9	J.B.	9	M	yes											yes	375	10	0
10	M.P.	5	M												yes	325	6	0
11	B.C.	16	F	yes										yes**	yes	75	18	Trace
12	M.C.	19	F	yes											yes	100	19	0
13	P.H.	9	F												yes	175	21	0
14	B.A.	5	F												yes	925	8	0
15	R.C.	7	M												yes	425	2	0
16	R.S.	13	M											yes**	yes	250	5	Trace

17	A.N.	13	F									yes	275	13	0
18	M.B.	7	F	yes								yes	375	5	0
19	R.G.	17	M									yes	225	12	1+
20	R.M.	9	M									yes	1325	3	0
21	J.P.	13	M									yes	75	2	0
22	J.M.	13	M	yes								yes	150	7	0
23	G.C.	11	M									yes	225	13	0
24	J.K.	11	F	yes								yes	475	28	0
25	F.A.	3	M	yes								yes	50	7	0
26	P.M.	8	M	yes								yes	500	11	0
27	R.L.	13	F									yes	150	5	0
28	J.C.	4	M	yes								yes	100	9	0

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* Murmur of mitral insufficiency or stenosis; stabilized or diminishing in intensity.

** Electrocardiogram not concomitant with other data.

was an only determination. Of the 28 ESRs only 17 were within the normal range, (0 through 10 mm. - male; 0 through 15 mm. - female), while eleven determinations were elevated above these limits. The highest ESR was 28 mm. There is a fair correlation between a negative CRP test and a normal ESR in inactive patients. When the ESR and the CRP test are compared for each patient, the results of both determinations coincide and suggest inactivity in seventeen patients, while they do not correlate in nine instances. The ESR is elevated in eight of these while its corresponding CRP is negative; the reverse of this, a positive CRP test and a normal ESR, occurs only once. In eight of these nine discrepancies, the ESR and the ASLO titers had smaller values than those of a few months previously. J.K. (No. 24) had a higher ESR but a lower ASLO titer. In the case of the one positive CRP with a normal ESR, the CRP had dropped to trace from 6-plus in seven months while the ESR and ASLO were returning to normal values. Apparently a negative CRP test more often reflects clinical inactivity, and the ESR may still be elevated when inactivity is suggested by all other data. Patients R.T., B.C., and R.G. (Nos. 2, 11, and 19), had both an elevated ESR and a positive CRP test although these patients were probably inactive.

Table III presents the findings in five rheumatic fever patients with activity not established. These met neither the criteria for rheumatic activity nor those for inactivity. Patients A.N. (No. 1) and R.L. (No. 3) would be classified as active by the original criteria of Jones (22) or Griffith (23), but under the modified classification (page 15) they did not meet the criterion of having at least one major manifestation, i.e. carditis, polyarthritis, chorea, subcutaneous nodules or "rheumatic" rash. All minor manifestations were

TABLE III. RHEUMATIC FEVER PATIENTS WITH ACTIVITY NOT ESTABLISHED - CLINICAL AND LABORATORY DATA

No.	Patient	Age	Sex	MAJOR CRITERIA								MINOR CRITERIA						
				HEART DISEASE *				Polyarthritis	Chorea	Subcutaneous Nodules	"Rheumatic" Rash	Fever	Arthralgia	Prolonged P-R Interval	Past History of Rheum. Fever	ASLO TITER	ESR	CRP TEST
				A	B	C	D											
1	A.N.	13	F									yes	yes	yes	yes	200	38	1+
2	B.G.	18	F					±				yes	yes			900	40	1+
3	R.L.	13	F									yes	yes	yes	yes	560	48	6+
4	R.G.	15	M	yes	yes									yes	yes	75	5	1+
5	S.L.	7	F						yes						yes	200	5	0

* A- Murmur of mitral insufficiency or stenosis; cardiac murmur stabilized or diminishing in intensity.

B- Cardiac enlargement relatively unchanged for one year.

present, however, including an elevated ESR and a positive CRP test. Although active carditis was not present according to these criteria, patient R.L. (No. 3) did have a pulse rate of 130 and heart sounds of poor quality. These two patients were most likely active and they were clinically managed as such. Patient B.G. (No. 2) had poorly defined arthritis on examination although polyarthritis was definitely present a month earlier. Cardiac murmurs were heard by several observers, but they were not felt to be murmurs of rheumatic activity. B.G. had fever, arthralgia, and an elevated ASLO titer and ESR. She had a 1-plus CRP test. She was probably active. Patient R.G. (No. 4) had the murmurs of mitral insufficiency and mitral stenosis and a prolonged P-R interval. Both had remained essentially unchanged for a period of at least one year. It is not obvious that active heart disease was present. The ASLO and ESR were within normal range, but there was a 1-plus CRP test. It is not clear just how R.G. should be classified, probably as inactive. Clinical management agreed with the classification of inactivity. Chorea was present in patient S.L. (No. 5); and other data reflected inactivity including the ASLO, CRP, and the ESR determinations. The ESR and the CRP test agreed in regard to activity or inactivity in four of these five patients with activity not established. In the one with disagreement, patient R.G. (No. 4), the ESR was 5 mm. with a 1-plus CRP test. He was managed as an inactive patient.

Table IV and V summarize the data of Tables I, II, and III. The CRP test and the ESR in 33 active, five with activity not established, and 28 inactive rheumatic fever patients are recorded. Thirty (91.9%) of the active cases had positive CRP tests. Thirty-two (97.0%) of

TABLE IV. THE RESULTS OF THE CRP TEST IN RHEUMATIC FEVER

CLASSIFICATION	NUMBER OF PATIENTS	CRP TEST POSITIVE	 NEGATIVE
ACTIVE	33	30	3
ACTIVITY NOT ESTABLISHED	5	4	1
INACTIVE	28	4	24

TABLE V. THE RESULTS OF THE ESR - WINTROBE - IN RHEUMATIC FEVER

CLASSIFICATION	NUMBER OF PATIENTS	ESR * ELEVATED	 NORMAL
ACTIVE	33	32	1
ACTIVITY NOT ESTABLISHED	5	3	2
INACTIVE	28	11	17

* Corrected to hematocrit of 47 mm. for males; 42 mm. for females. Normal range: 0 through 10 mm. for males; 0 through 15 mm. for females.

these same cases had an elevated ESR. Of the 28 patients in the inactive group, 24 (85.7%) had a negative CRP test, while only seventeen (60.7%) had an ESR within the normal range. The patients with activity not established have just been discussed in the preceding paragraph.

The CRP test results during menstruation are summarized in Table VI. Thirty-one tests were negative of the 32 performed. There is apparently no C-reactive Protein present in the serum of females during or near the menstrual period. The one test that was positive was in a trace amount. This patient reported a gastrointestinal upset the day prior to the drawing of this sample.

The results of the ESR during menstruation are somewhat less clear. (Table VII) Eight of the 28 ESR determinations showed elevations above 15 mm. when all tests were corrected to a hematocrit of 42 mm. while 20 of the erythrocyte sedimentation rates were within normal limits.

The CRP test results and related clinical and laboratory data in eighteen patients with upper respiratory infections are found on Table VIII. The ages represented in this group were four through sixteen years. Eleven were males and seven were females. One degree Fahrenheit was added to oral temperatures to convert each to the corresponding rectal temperature. The findings on physical examination were graded 1-plus, 2-plus, 3-plus, 4-plus. Tend. in the Cervical Glands column refers to tender lymph nodes on palpation, N. flora denotes normal flora in the Nose and Throat Culture column. The CRP test was positive in eleven instances and negative in seven. The ESR was elevated in eleven and within the normal range in five. In eleven patients the ESR and the CRP were correlated; five did not agree. The four

TABLE VI. THE C-REACTIVE PROTEIN TEST RESULTS DURING MENSTRUATION

SAMPLE	NUMBER OF DETERMINATIONS	CRP TEST POSITIVE	CRP TEST NEGATIVE
PREMENSTRUAL (1-2 Days)	3	0	3
MENSTRUAL (1-7 Days)	22	1	21
POSTMENSTRUAL (1-2 Days)	7	0	7
TOTALS	32	1	31

TABLE VII. THE RESULTS OF THE ESR - WINTROBE - DURING MENSTRUATION

SAMPLE	NUMBER OF DETERMINATIONS	ESR TEST * OVER 15 mm.	ESR TEST * 0-15 mm.
PREMENSTRUAL (1-2 Days)	2	0	2
MENSTRUAL (1-7 Days)	19	5	14
POSTMENSTRUAL (1-2 Days)	7	3	4
TOTALS	28	8	20

* Corrected to a hematocrit of 42 mm.

TABLE VIII. UPPER RESPIRATORY INFECTIONS - CLINICAL AND LABORATORY DATA

No.	Patient	Age	Sex	Day of Illness	Rectal Temperature	Otitis Media	Rhinitis	Pharyngitis	Laryngitis	Cervical Glands	Cough	N. and T. Cultures	WBCs x1000	ESR	CRP Test
1	T.A.	14	M	2	101.6			+	++	++ Tend.	++	Pneumos.	11.6	3	1+
2	J.D.	13	M	14	100.0	+	++	++		++ Tend.	+	Hem. strep.	14.6	26	3+
3	M.F.	7	F	7	100.6			+		++	+	N. flora	23.6	10	0
4	L.G.	8	M	7	100.0		!	+		+		N. D.	4.2	11	1+
5	R.G.	12	M	60	100.0		++	+		++ Tend.	++	N. D.	N.D.	19	0
6	A.H.	14	M	4	100.4		++	++		+++ Tend.	+	N. D.	5.5	6	2+
7	B.H.	8	F	14	101.6			+		+	++	N. flora	10.0	28	0
8	H.L.	14	M	2	104.0		++	++		++++	+	N. flora	6.0	3	0
9	J.M.	8	M	3	100.2	+	+	++		++ Tend.	+	N. D.	12.0	35	2+
10	A.M.	4	M	7	104.6		++			+	++	N. D.	14.3	12	2+
11	B.M.	6	F	3	100.6	+	+			+	+	N. D.	5.0	27	2+
12	E.O.	11	F	14	100.0					++	+	N. D.	N.D.	N.D.	3+
13	R.P.	15	M	21	100.0	++				+++ Tend.		N. D.	N.D.	10	0
14	J.T.	7	M	8	99.8			+		+		N. flora	6.9	17	0
15	C.S.	16	F	4	99.6		+	+				N. flora	N.D.	34	2+
16	S.L.	12	F	5	103.0			++		++ Tend.		Hem. strep.	15.0	17	1+
17	N.C.	16	F	3	102.0			+				Pneumos.	4.9	21	6+
18	E.J.	10	M	10	103.8		+	+		+	+	N. D.	6.0	N.D.	0

! Nose packed with gauze.
Tend. - Glands tender on palpation.

N.D. - not done.
N. flora - normal flora

patients from whom pneumococcus or hemolytic streptococcus organisms were cultured had positive CRP tests. The CRP test was positive in nine of eleven patients who were within seven days of the onset of their illnes; in seven patients ill for eight days or more, only two had a positive CRP test. CRP tests were positive in seven of ten patients with fever (over 100.0°, rectal); but also positive in four of eight who were afebrile. There was no other apparent correlation with a positive CRP test. Apparently mild upper respiratory infections are a sufficient stimulus to cause the production of C-reactive Protein in well over half of the patients. Most of these patients on examination had a mild fever and were somewhat more ill than if they had merely a "common cold". It would be interesting to know if a cold by itself would produce a positive CRP test.

Table IX presents the results of the CRP test in 36 pregnant females seen in the Grace-New Haven Community Hospital. There were thirty negative and six positive results. Of the six tests that were positive, five were from patients in the last trimester of pregnancy. Patient, M. E., who had a 1-plus CRP test, had cervicitis with a moderate amount of leukorrhea. She was otherwise well. Patient E. T., had two CRP tests performed. The first was done 40 days prior to delivery; it was negative. The blood for the second test was drawn during labor; it was 2-plus. At this time, however, she also had an upper respiratory infection characterized by a post-nasal drip and subacute bronchitis which may have produced the positive test. All other patients were apparently healthy normal pregnant women. Six in the "last trimester" were actually in labor when the blood was drawn.

TABLE IX. THE RESULTS OF THE C-REACTIVE PROTEIN TEST IN PREGNANCY

PATIENT	AGE	TRIMESTER	CRP TEST	PATIENT	AGE	TRIMESTER	CRP TEST
S.B.	25	1st	0	E.C.	24	3rd	0
G.B.	27	1st	0	M.T.	15	3rd	0
I.F.	27	1st	0	J.B.	30	3rd	Trace
J.M.	37	1st	0	E.P.	28	3rd	0
H.M.	37	1st	0	D.P.	27	3rd	0
R.W.	33	1st	0	E.T. **	16	3rd	2-plus
A.M.	23	1st	0	A.R.	25	3rd	0
J.H.	25	1st	0	B.P.	18	3rd	1-plus
E.F.	36	2nd	0	M.G.	31	3rd	0
L.H.	22	2nd	0	T.L.	29	3rd	0
S.H.	19	2nd	1-plus	H.S.	26	3rd	0
B.M.	21	2nd	0	J.A.	26	3rd	0
D.P.	34	2nd	0	E.C.	30	3rd	Trace
M.R.	29	2nd	0	J.L.	38	3rd	0
E.B.	17	3rd	0	M.L.	25	3rd	0
M.E. *	23	3rd	1-plus	J.R.	23	3rd	0
C.B.	24	3rd	0	L.H.	32	3rd	0
E.T.	16	3rd	0	B.C.	23	3rd	0

* M.E. had cervicitis.

** E.T. had bronchitis; an earlier determination done 40 days previously was negative.

SUMMARY

TRIMESTER OF PREGNANCY	NUMBER OF DETERMINATIONS	CRP TEST POSITIVE	CRP TEST NEGATIVE
FIRST	8	0	8
SECOND	6	1	5
THIRD	22	5	17

Schieffelin and Company have indicated that plasma should not be used for the CRP test (37). Because plasma samples may be available when serum is not, a study of the possible use of plasma for the CRP test was made. The results are found on Table X. A sample of serum and a sample of plasma were obtained from fifteen patients. The serum samples were positive for CRP in five instances and the corresponding plasma samples were also positive. Three of these were more strongly positive in the plasma than in the serum. Seven serum samples were negative and the seven corresponding plasma samples were also negative. In three other instances the serum samples were negative while a trace amount of precipitate occurred in the plasma. Generally, the plasma samples were more difficult to read inasmuch as the precipitate was scattered. A cloudiness was present occasionally which made reading more difficult. It is probable from this study that the CRP test can be performed with plasma, although it would appear to be more accurately determined with serum.

TABLE X. THE RESULTS OF THE CRP TEST WITH PLASMA AND WITH SERUM

PATIENT NUMBER	PLASMA	SERUM
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	Trace !	0
9	Trace !	0
10	Trace !	0
11	1-plus	1-plus
12	2-plus	2-plus
13	3-plus	2-plus
14	4-plus	3-plus
15	4-plus	2-plus

! Difficult to read due to a peculiar cloudiness.

VI. DISCUSSION

Rheumatic fever has long been considered a serious illness primarily because of the severe cardiac lesions which frequently follow in the wake of this disease. The diagnosis of activity or inactivity in rheumatic fever patients is not always easily done. Investigators in many fields have searched for methods to make this evaluation more certain. To date no single laboratory technique is as valuable as total clinical judgment. Many methods and tests have been developed, however, to assist the clinician with this problem. Some of these are the ASLO, the ASK, and the AH titers; the WBC count; the P-R interval of the electrocardiogram; and the erythrocyte sedimentation rate.

Recently the C-reactive Protein (CRP) test has also been advocated as a reliable indication of rheumatic activity (39, 49). Certain investigators have stated that this test is the most sensitive of all laboratory aids in the diagnosis of rheumatic activity or inactivity. A positive test according to these workers is found in nearly all active rheumatic fever patients, and a negative test in most of the inactive patients.

The CRP test is not specific for rheumatic fever, on the contrary, it is a very non-specific indicator of inflammation or tissue destruction. A positive test is found in the serums of most patients with viral, bacterial, collagen, and many neoplastic diseases, and yet this protein is never present in the serum of a normal healthy patient (4, 17, 16, 32, 48). There is a good possibility that some minor conditions such as mild upper respiratory infections, sinusitis, pharyngitis, and asthma may affect it (11, 16, 22). If so, certainly the value of the test in any specific illness, such as rheumatic fever would be limited. All possible

sources of positive tests must be known and ruled out if this is to be a valuable aid to the clinician in rheumatic fever.

This investigation was undertaken to evaluate the CRP test more fully in rheumatic fever patients by a comparison of the CRP test with the ESR for active and inactive patients, and to determine whether or not commonly present minor conditions such as upper respiratory infections, menstruation, or pregnancy will affect the CRP test. Determinations for C-reactive Protein were done using a very sensitive rabbit antiserum in a simple precipitin test (37, 41). For comparison the Wintrobe method of determination and correction of the ESR was used. The normal range for the ESR was: 0 through 10 mm. for males; 0 through 15 mm. for females (45, 46, 47).

The criteria for the diagnosis of rheumatic fever included: carditis, polyarthritis, chorea, subcutaneous nodules, and a "rheumatic rash" as major manifestations; and fever, arthralgia, prolonged P-R interval, past history of rheumatic fever, elevated ASLO titer, and an elevated ESR as minor manifestations. (Note Appendix and page 15). The results of this investigation showed that the CRP test was positive in over 91% of 33 *active* rheumatic fever patients. The ESR, by comparison, was elevated in over 95% of *active* cases. The inactive group had positive CRP tests in 85% of 28 patients, while the ESR was within normal range in only 60% of patients studied. The CRP test results in patients with *undetermined rheumatic activity* correlated with the clinical condition in for of five patients in this group. According to this study as well as others (4, 39, 49), *the CRP test is positive at least as often as the ESR is elevated in clinically active rheumatic fever patients. Among the inactive, however, the CRP appears to reflect the clinical diagnosis*

more frequently than does the ESR. Anderson and Stollerman have shown that there is an excellent correlation of the CRP test with the clinical condition and that the CRP test gives the first indication of clinical improvement when it is compared with the ESR, ASLO, and WBC. The ESR is rather slow in returning to normal levels according to these workers (4, 39). This is also indicated by the present study. The slow return of the ESR to normal and the rapid change to negative of the CRP test probably explain why there was a difference in percentages for the inactive group. An early return to normal levels of one laboratory determination is not necessarily an indication of recovery. And we are not certain that the slow return of the ESR is merely a lag phenomenon. Serial studies mentioned do, however, show an excellent correlation between a negative CRP test and a good prognosis.

This investigation has shown that common upper respiratory infections such as rhinitis, pharyngitis, or otitis media, will incite alpha globulin production and a positive CRP test. In eleven of 18 patients in this study the CRP test was positive. More positive tests occurred in the early phase of illness. The test was positive for afebrile as well as febrile patients; it was positive when the WBC count was within normal limits. Indications are that a patient who has a significant upper respiratory infection may well have a positive CRP test, and the clinician must be aware of this likelihood in his interpretation of a patient with rheumatic fever.

The pregnancy group was not as clear. Of 36 determinations, thirty were negative. Five of the six positive tests were from women who were in the last trimester of pregnancy; two of the positive tests were from women who were actually in labor. Two positive tests may have been attributable to mild infections which were also present.

The results are equivocal for the last trimester but quite definite for earlier in pregnancy, that is, the CRP test may be positive in the later stages of pregnancy and negative in the earlier portion. This agrees in part with the work of Shetlar (35) who found ten of 27 patients in the last trimester of pregnancy who had positive CRP tests. Hedlund, by contrast, studied 25 patients from the second to the tenth month of pregnancy and found none that had positive CRP tests. He used the earlier and less sensitive technique of pneumococcal capsular swelling (16). Other commonly present conditions which might also confuse the interpretation of this test in rheumatic fever are eczema, dental caries, allergy, the "common cold", and early phases of certain neoplastic diseases.

The CRP test in this study was negative in menstruation in 31 of 32 instances. The one test that was positive was merely a trace. A gastrointestinal upset may have caused this. Certainly these results would indicate that a menstruating rheumatic fever patient should not have a positive test due only to the menstruation.

In comparing the CRP test with the ESR as was done in rheumatic fever, upper respiratory infections, and in menstruation; certain differences in the two tests were brought out which complicated the evaluation of the ESR particularly. There are several different methods of performing the ESR--Wintrobe, Westergren, Cutler, etc. Each of these methods gives somewhat different results although there is an excellent correlation among them with some exceptions. Hollinger stated that the three methods mentioned have a correlation of 0.90 (9, 10, 18). There is a great deal of discussion in the literature concerning correction of the sedimentation rate for the packed cell volume (18, 46). Many of these problems must be considered in a

fairly rigid comparison of the ESR with the CRP test. There is no definitely established normal range for children of rheumatic fever age, and some investigators state that it varies with specific ages as well as sex. There are many factors which may affect the sedimentation rate: fibrinogen, gamma globulin, hyaluronic acid, large-asymmetrical moleculee of many types, albumin, hormone therapy, acetylsalicylac acid, liver disease, temperature, slight tube inclination (8, 13, 29, 30, 33, 47). It would be of real value if acceptable standardized normal ranges could be definitely established. Certainly the ESR would then be more useful and valid than it is at present.

The CRP test appears to be less complicated to interpret. There are no correction factors needed, no normal ranges to interpret, and as far as is known a positive test is dependent upon only one substance --alpha globulin--for which highly potent and specific antiserum is readily available. The elevation of alpha globulins in inflammation has been well-documented by electrophoretic studies (24, 31, 43). According to this study, there are instances of negative tests in active rheumatic fever patients. Whether this is due to errors in diagnosis or to the presence of some blocking mechanism in the serum is not known. Certainly hormone therapy will cause a diminution of the CRP as well as the ESR, but Good has shown that this is most likely due to improvement of the clinical condition rather than due to a specific effect of the hormone on CRP production (11). A distinct advantage of the CRP test is that it is strongly positive in congestive heart failure secondary to rheumatic fever; the ESR may drop to low normal values in this condition.

The CRP test is now being investigated further in viral, bacterial, collagen, neoplastic and other diseases (4, 7, 16, 32, 48). Generally the test is positive in all of these conditions during the acute phase of the illness, and the test becomes negative in the early convalescent stage. Some have suggested that the CRP test may be of assistance to the clinician in helping to differentiate psychological from organic illnesses. Whether anxiety neuroses, and schizophrenia, for example will produce a positive CRP test has not been ascertained. Rantz has very recently given a paper on a study of 400 hospital patients. He used CRP antiserum for CRP determinations. His studies included collagen, bacterial, ~~and~~ viral and neoplastic diseases. Positive results were found in most instances. He suggested that the test had great promise as a mass screening test and that false positives in healthy persons were extremely rare. In fact he feels that any positive test should be considered indication of pathology until proven otherwise. He reiterated what has been said here about the problems involved in sedimentation rate correction and normal value (32).

The C-reactive Protein test is a valuable aid to the diagnosis of rheumatic activity. Other sources of positive tests, however, must be known and ruled out before the assumption is made that a positive test is due to an exacerbation of rheumatic fever. Certainly this test, as others before it, should only be used in conjunction with all other means available in the diagnosis of rheumatic fever.

VII. SUMMARY

1) Sixty-six rheumatic fever patients were classified as *active*, *activity not established*, and *inactive*. An evaluation of the CRP test in these patients was made by comparing the results with those of the erythrocyte sedimentation rate. Over 90% of the active patients had positive CRP tests, and 95% had an elevated ESR. Of the inactive group, 85% had negative CRP tests while only 60% had normal ESRs. In patients with *activity not established* the CRP test and the ESR were correlated in four of five patients studied. This investigation agrees with others in declaring the usefulness of the CRP test as a diagnostic aid in rheumatic fever. This test has the advantage over the ESR in that it requires no correction factors, and there is no normal range to interpret.

2) Two commonly present conditions may complicate the evaluation of the CRP test in rheumatic activity. It was found that upper respiratory infections and possibly the *last trimester* of pregnancy can produce positive CRP tests. In this study eleven of 18 patients with mild upper respiratory infections, and six of 36 pregnant patients had C-reactive Protein in their serums. By contrast, menstruation and the *early months* of pregnancy almost certainly do not produce positive CRP tests. Of 32 menstrual patients 31 had negative CRP tests. And 13 of 14 pregnant patients in the first and second trimester were negative.

3) A review of the literature was made in which various methods for CRP determination and the range of usefulness of the CRP test were described.

VIII. APPENDIX

Jones Criteria (Modified) for Guidance in the Diagnosis of Active Rheumatic Fever in Process of Consideration by American Council on Rheumatic Fever and Congenital Heart Disease*

In 1944, the late Dr. T. Duckett Jones published criteria for the diagnosis of rheumatic fever which have been generally accepted by the practicing physician and the research worker in the United States and in many parts of the world. In 1950, Dr. Jones guided the development of revision of his criteria for research purposes and these were used in the U.K.-U.S. Cooperative Study on "The Relative Effectiveness of ACTH, Cortisone and Aspirin in the Treatment of Rheumatic Fever." Just prior to his death, he participated in a conference on the revision of his original data for use by the practicing physician. These modified criteria are based in great measure upon his suggestions for the revision of his original criteria.

Rheumatic fever is frequently related to previous infection with the group A beta hemolytic streptococcus, but the mechanism of the disease is unknown. The boundaries of this disease are indefinite and its differentiation from other diseases of connective tissue is sometimes impossible. There is no specific laboratory diagnostic test. Criteria for diagnosis must therefore be arbitrary and empirical. In their establishment an attempt is made to characterize the core of the problem, that is, to identify the group of individuals in whom there is a high probability of having prolonged acute illness with involvement of the heart, and the added hazard of death during the acute attack or the eventual development of chronic valvular heart disease.

Criteria are necessary because the diagnosis of this disease must never be made by exclusion. The tendency to label as rheumatic fever a chronic febrile disease for which no obvious cause can be found is

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* Unpublished data - revision of April 15, 1955

to be deplored. The tragedy of invalidism which may lie in the wake of a false diagnosis of rheumatic fever in a normal individual may be even greater than the possible harm of a mis-diagnosis in questionable cases. However, both overdiagnosis and underdiagnosis will be minimized by using acceptable criteria, arbitrary as they may be. The effectiveness of prophylactic regimens requiring prolonged administration of sulfadiazine or antibiotic agents places a grave responsibility on the physician in the diagnosis of this illness.

As originally proposed by Jones, the diagnostic features of this disease are divided into major and minor categories dependent upon their relative occurrence in rheumatic fever and among other diseases syndromes from which this disease must be differentiated. Thus chorea is included among the major criteria while fever, a symptom common to many diseases, is placed in a minor category. These major and minor categories have no significance beyond their diagnostic import, either as to prognosis, or amount of "rheumatic activity" or severity of acute illness. Indeed, a severe manifestation of rheumatic fever such as rheumatic pneumonia is not included at all because it is difficult and sometimes impossible to differentiate from congestive cardiac failure and because it almost always occurs in patients whose disease is so obvious as to offer no difficulty in diagnosis.

As originally proposed, the presence of two major manifestations or one major and two minor manifestations indicates (with one notable exception, see page 6) a high probability of the presence of rheumatic fever. Different from the previous recommendations but similar to the 1950 revision is the division of the original minor criteria into two groups, one of <u>minor manifestations</u> to be used in the recommended formula, and <u>other manifestations</u> of rheumatic fever (including constitutional manifestations) which may be used to support the diagnostic

impression provided by the combination of major and minor criteria. Again, this division is an arbitrary one with the recognition that rarely a seriously ill patient with this disease will have little more than constitutional manifestations. But these criteria are not meant to substitute for the wisdom and judgment of the clinician. They are designed only to guide him toward a positive diagnosis of the disease with the suggestion that he follow carefully all questionable cases and restrict the use of the label of rheumatic fever to those whose illness meets acceptable criteria.

(Major and minor criteria follow on the
following few pages).

Major Criteria

The major significant manifestations are listed and defined as follows:

1. CARDITIS - as evidenced by any one of the following:
   a. The appearance of a significant apical systolic murmur*, apical mid-diastolic murmur**, or basal diastolic murmur*** in an individual without a history of rheumatic fever or pre-existing rheumatic heart disease, or a change in the character of any of these pre-existing murmurs under observation in an individual with previous history of rheumatic fever or rheumatic heart disease.
   b. Obvious increasing cardiac enlargement by x-ray.

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* A significant systolic murmur is blowing and long - filling most of systole, is heard best at the apex, and is as well transmitted toward the axilla as over the precordium and does not change with position or respiration. It must be differentiated from an innocent (functional) murmur which is frequently found in normal people. This is a systolic, occasionally harsh murmur heard best along the left sternal border but which usually changes with position and respiration. Borderline systolic murmurs, intermediate in location and nature, occur and should be carefully watched. In the absence of other cardiac disease, e.g., congenital septal defect, questionable murmurs which are intermittently present or which, after a period of observation, cannot be clearly classified as significant, are rarely of any import.

** A significant organic apical systolic murmur is frequently accompanied by a low-pitched, short mid-diastolic murmur which is sharply localized to the chest wall over the apex of the heart and often heard best with a patient in the left lateral position with the breath held in expiration. This murmur, rarely present in the absence of an apical systolic murmur, confirms the significant nature of the latter. It must be differentiated from the long, low-pitched, crescendo apical presystolic murmur followed by an accentuated mitral first sound which is indicative of mitral stenosis, i.e., old rheumatic heart disease, suggesting previous rheumatic fever but not acute carditis.

*** The development of a basal diastolic murmur of aortic insufficiency is also indicative of carditis. It is an early, short, diminuendo murmur usually heard only or heard best along the left sternal border in deep expiration. It has great diagnostic value, even though it may be difficult to hear and present only intermittently.

c. Pericarditis manifested by a definite friction rub or pericardial effusion.

d. Congestive heart failure (in a child or young adult under 25) in the absence of other causes.

2. POLYARTHRITIS - Polyarthritis tends to be migratory and is manifested by pain <u>and</u> limitation of active motion or tenderness, heat, redness or swelling of two or more joints, Arthralgia alone without objective evidence of joint involvement is not a major manifestation.

3. CHOREA - This occurs more frequently among females, is rarely found after adolescence, and must be differentiated from habit spasm. The movements should be of at least moderate severity to justify its use as a major criterion of diagnosis.

4. SUBCUTANEOUS NODULES - Subcutaneous nodules are shot like, hard bodies felt over the extensor surface of certain joints, particularly elbows, knees and wrists, and over the spinous processes of the thoracic and lumbar vertebrae and the scalp. These are useful in chronic cases because they usually tend to occur late in the course of the disease. They must not be confused with the nodules of erythema nodosum which are red, hot and usually appear over the anterior surfaces of the tibiae, and which may be associated with recent streptococcal infection, rheumatic fever or tuberculosis.

5. ERYTHEMA MARGINATUM - This transient, recurrent, pinkish, characteristic rash of rheumatic fever in which the color gradually fades away from its sharp scalloped edge, is found mainly over the trunk, sometimes on the extremities, but never on the face. This rash is extremely transient, is brought out by heat and migrates from place

to place hour by hour. Although sometimes confused with other rashes, particularly erythema multiforme, its appearance is so distinctive that once clearly seen by the clinician, it is easily recognized again.

Minor Criteria

1. FEVER - A significant rise in temperature is a common symptom but because it occurs in so many illnesses, it has little differential diagnostic value and is included only as a minor criterion. In order to be included, the elevation in temperature must clearly exceed the normal diurnal fluctuation in which there is great individual variation.
2. ARTHRALGIA - Pain clearly located without objective findings is only a minor criterion for diagnosis. The pain must be in the joint not in the muscles or other periarticular tissues and must be distinguished from the frequent, nocturnal manifestation in normal children of severe pain in the extremities. Arthralgia must not be used as a minor manifestation when polyarthritis is included as a major manifestation.
3. PROLONGED P-R INTERVAL IN THE ELECTROCARDIOGRAM - Since this manifestation may be non-specific, it is considered a minor manifestation and is not diagnostic of carditis. It cannot be used if carditis is already included as a major manifestation.
4. INCREASED ERYTHROCYTE SEDIMENTATION RATE OR PRESENCE OF C-REACTIVE PROTEIN OR LEUKOCYTOSIS - Elevation in any of these non-specific tests may be included as a minor manifestation. Particularly to be deplored is the tendency to use any of these tests as a major criterion or as diagnostic of acute rheumatic fever. There are many other non-specific tests, but these three are most commonly used.

5. EVIDENCE OF PRECEDING BETA HEMOLYTIC STREPTOCOCCAL INFECTION - This must be documented by, (a) a history of scarlet fever or by a typical, clinical picture of other streptococcal infection preceding the onset of rheumatic fever by one week to one month, the latter confirmed by a history of immediate contact with other individuals having typical streptococcal infection or by positive culture of the nose and throat in which the beta hemolytic streptococcus predominates, or (b) by an elevated antistreptolysin C-titer or preferably by a rising titer.
6. PREVIOUS HISTORY OF RHEUMATIC FEVER OR THE PRESENCE OF OLD RHEUMATIC HEART DISEASE - The existence of either of these may be used as a minor criterion for diagnosis of active infection but the previous history must be documented by the same objective criteria as are used for the diagnosis of the present attack or by the presence of rheumatic heart disease.

Other Manifestations

These include constitutional manifestations such as loss of weight, easy fatigability, malaise, sweating, pallor or anemia, and other manifestations such as a family history of rheumatic fever, epistaxis, precordial pain, abdominal pain, elevated sleeping pulse rate, tachycardia out of proportion to fever, and headache. These are additional evidence of the presence of rheumatic fever but are not to be included in the diagnostic criteria.

Certain combinations of major and minor manifestations cannot be used because each represents the same aspect of the disease. Thus, arthralgia may not be used as a minor in the presence of polyarthritis as a major, the P-R interval is not to be counted in the presence of carditis and not more than one of the non-specific laboratory tests

(sedimentation rate, C-reactive protein or leukocyte count) is to be included as a minor.

There are combinations of these diagnostic criteria which occur in the presence of other illnesses which must be ruled out before a definitive diagnosis is made. One combination in particular, polyarthritis, fever, and elevated sedimentation rate, is the weakest of all the combinations of major and minor criteria. The diseases to be ruled out include rheumatoid arthritis, gonococcal arthritis, lupus erythematosus disseminata, subacute bacterial endocarditis, leukemia, sickle cell anemia, Still's disease, serum sickness (including that associated with penicillin sensitivity), tuberculosis, anterior poliomyelitis, undulant fever, and septicemias, particularly meningococcic.

These revised criteria for the diagnosis of rheumatic fever are presented with the knowledge that they are tentative and must be modified as new information on rheumatic fever is accumulated.

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